Collink·3D 50

Human Collagen BioInk

Cat# W10199

Introduction

Collagen is a structural protein that serves as the basic building block of the extracellular matrix in the human body. Its natural structure, inherent biocompatibility, substantial clinical practice and biodegradability render it an ideal biomaterial for regenerative medicine.

CollPlant has developed Collink.3D™ 50, the first and only human collagen bioink, based on plant-derived recombinant human type I collagen (rhCollagen) that is mass produced at high consistency.

The product is compatible with major 3D bioprinting technologies and cell types. Collink.3D™ 50 enables scalable and reproducible biofabrication of scaffolds, tissues and organs for 3D modeling as well as transplantation purposes, while ensuring perfect recapitulation of the properties of native tissues and organs. Biofabricated constructs composed of Collink.3D™ 50 offer superior biological performance, consistency and safety.

Description

Collink.3D™ 50 is rhCollagen methacrylamide, to be used as a curable bioink platform for biofabrication applications including 3D bioprinting.

RhCollagen, produced from genetically engineered tobacco plants, was modified by reacting its free amines, i.e., the α-amino groups of the lysine residues as well as the α-amino groups on the N-termini. As a result, approximately 50% of the total primary amines of the collagen molecule were converted into methacrylamides. Following modification, the protein was further purified then concentrated to yield the final product in 10 mM HCl solution.

Intended use

Collink.3D™ 50 is intended for the biofabrication of tissue and organ models, including 3D cell cultures, as well as scaffolds, tissues and organs for implantation.

Biofabricated constructs composed of Collink.3D™ 50 can be used in a wide range of tissue model applications including drug discovery, drug screening, disease modeling and tissue testing. In addition, it can be used for the development and manufacture of transplantable tissues, scaffolds and organs with complex architectures, to meet specific properties.

Collink.3D™ 50 is compatible with major printing technologies, e.g., extrusion, inkjet, photolithography, and laser-induced forward transfer (LIFT).

The Collink.3D™ 50 is for R&D use only and is not intended for human use.

Characterization and Testing

General

Collink.3D™ 50 is a highly homogenous solution of intact, plant-derived triple-helical human type I collagen, rich in cellular binding domains and free of tissue residues. The product has demonstrated a high safety profile.
Collink.3D™ 50 has the following characteristics as shown in Table 1.

Table 1: Characteristics of Collink.3D™ 50

Parameter	Specification
pH	1.9 – 2.4
Appearance	Clear, transparent, no visible particles
Identity	Type I collagen
Purity	> 95% Type I collagen
Concentration	13 – 17 mg/mL
Degree of Functionalization#	45-65%
Total Aerobic Microbial Counts	< 10 CFU/mL
Total Yeast and Mold Counts	< 10 CFU/mL
Endotoxins	< 7 EU/mL
Heavy metals (Elemental analysis)	< 5 ppm
Curing Kinetics- Time to G' >100 Pa	≤ 5 sec

Determined using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) assay, relatively to the rhCollagen source.

Structural identity [1]

The SDS-PAGE gel image (Figure 1) shows distinct bands at ~100 kDa correspond to the characteristic mixture of α1 and α2-chains, with the expected slight shift in weight of the modified molecules (lanes #3 and #4) vs. the native molecule (lane #2).

The absence of low-molecular-weights fractions demonstrate the high protein purity.

Collagen exhibits a characteristic circular dichroism (CD) spectrum with a positive peak at ~220 nm and a negative peak at ~198 nm, indicative of its intrinsic secondary structure and folding properties (Figure 2).

The CD spectrum of Collink.3D™ 50 indicates that the chemical modification of the molecule did not result in perturbation of the overall conformation, and that rhCollagen preserves its native structure and structural integrity.

The typical ratio of the intensity of the positive peak near 220 nm over the intensity of the negative peak near 198 nm (Rpn) values, used to determine triple-helix content, are 0.118 and consistent with fully folded triple helices.

Mechanical properties [2]

Due to its high concentration, Collink.3D™ 50 product can be easily incorporated to form multicomponent compositions. It can be mixed with other polymers (synthetic, natural) and ECM-based additives to develop compositions with well-controlled properties.

It is designed to provide hydrogel compositions with mechanical and rheological properties, that can be controlled by collagen concentration, crosslinking conditions, and the nature of the additives.

Collink.3D™ 50 does not form a gel at room temperature, which makes it easy to handle and use. It features a shear-thinning profile (characteristic curve is presented in Figure 3A), that can be controlled by concentration adjustment and additives.

Collink.3D™ 50 maintains the physical and biological properties of its non-modified collagen source while allowing crosslinking at various light wavelengths using different photoinitiators. Collink.3D™ 50 shows remarkably fast curing kinetics.

Figure 3B demonstrates the curing capability of a 10 mg/mL solution of Collink.3D™ 50 in 10 mM HCl using 0.1% Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as a photoinitiator. After initially attaining G'> 100 Pa within 2.5 seconds, the material reaches a plateau G' value of 1200 Pa.

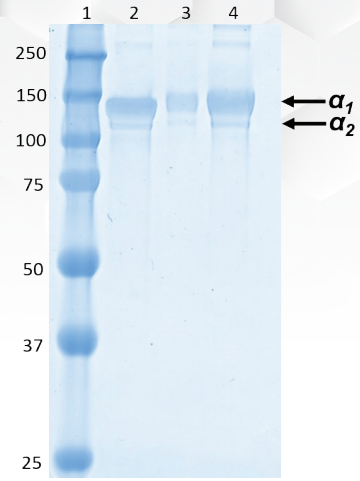

Figure 1: Lane 1: Standard protein marker; Lane 2: Recombinant human type I collagen source for Collink.3D™ 50; Lane 3 and 4: 5 µl and 25 µl sample of Collink.3D™ 50, respectively.

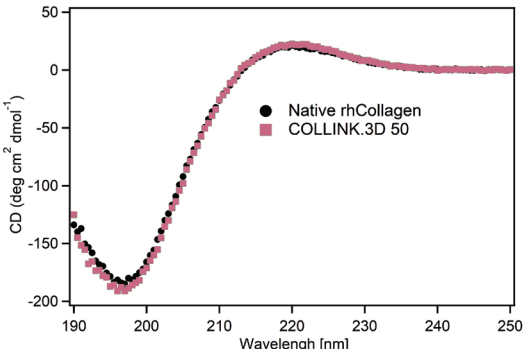

Figure 2: Typical CD spectra of Collink.3D™ 50 (pink) and its rhCollagen source (black) at 0.3 mg/mL in a 10 mM HCl solution.

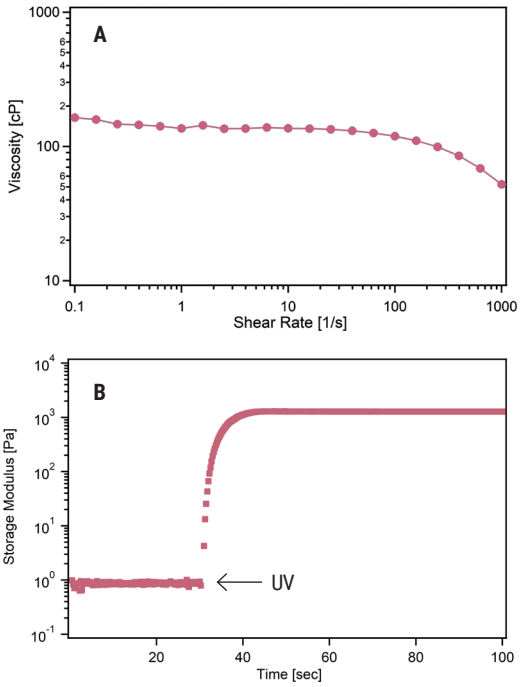

Figure 3: Rheological characterization of a 10 mg/mL Collink.3D™50 solution in 10 mM HCl. A: Viscosity measurement using the rotation flow sweep test method. B: Curing kinetics measurement at 365 nm irradiation (50 mW/cm^2), using the oscillation time sweep test method and 0.1 % LAP as a photoinitiator.

Figure 4 shows typical gel stiffness measured by applying compression stress on a crosslinked Collink.3D™ 50 discoid-shaped construct, fabricated from a 10 mg/mL solution in 10 mM HCl containing 0.1% 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) as a photoinitiator. The compression curve demonstrates a compression modulus of 26 kPa, with sample failure at 36% strain and 156 kPa stress.

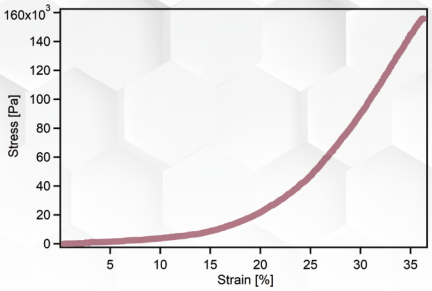

Figure 4: Typical compression stress-strain curve of a disc-shaped Collink.3D™ 50 construct, prepared from 1.6 mL Collink.3D™ 50 exposed to 365 nm irradiation for 2 min.

Cytocompatibility [3]

Collink.3D™ 50-based bioinks are biocompatible materials that enable the biofabrication of constructs with distinct properties that support adhesion, proliferation and function of different cell types and enhance rapid tissue repair. Collink.3D™ 50 can be used for both cell embedding and post-curing cell seeding. The low bioburden renders it compatible with long-term *in-vitro* studies, with no risk of contamination. Formulations based on Collink.3D™ 50 support culture of various cell types, including fibroblasts, endothelial, epithelial, stem and cancer cells. As shown in Figure 5, cells, mixed with Collink.3D™ 50 before printing or seeded onto biofabricated 3D scaffolds, showed superior cell adhesion and proliferation compared to bioink formulations lacking Collink.3D™ 50, with cell viability commonly exceeding 90%. Cells exhibited significantly improved performance, as manifested by cell spreading, well-organized cytoskeletal structures and cell-to-cell interactions. Moreover, Collink.3D™ 50-based bioinks have also been shown to support microvascular networks formation and maturation.

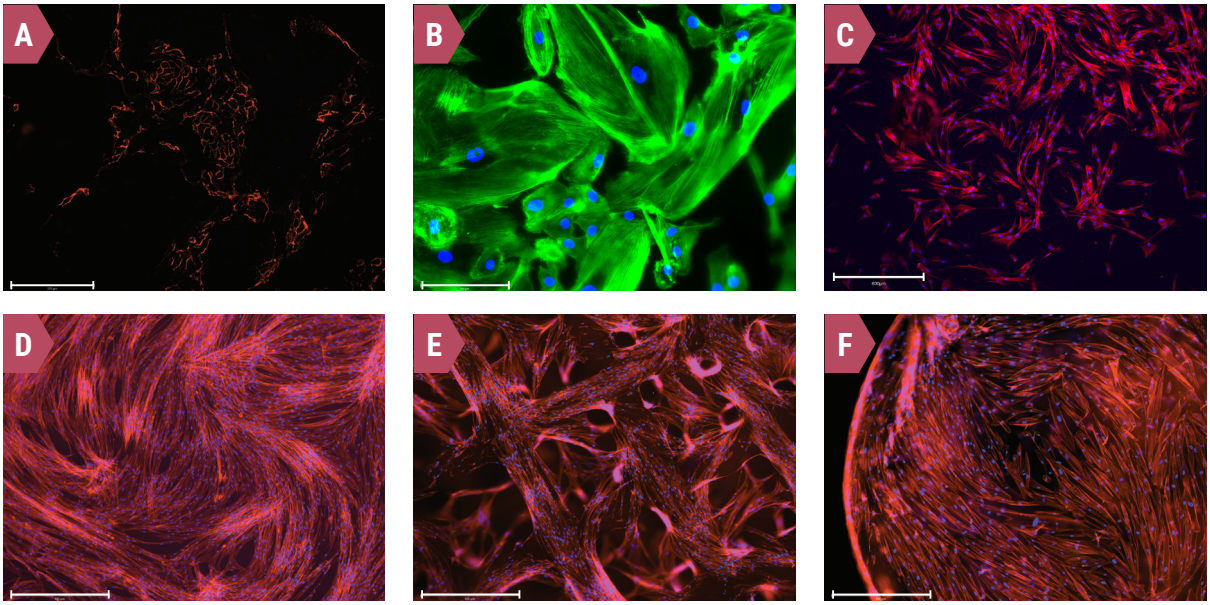

Figure 5: Fluorescent micrographs of cells grown on Collink.3D™ 50 layer [A-C] and 3D-printed constructs [D-F]. CD31 endothelial membrane specific staining of cells after 6 days in culture [A] (10X objective, scale bar: 275 µm). Human endothelial cells stained with DAPI (nuclei) and GFP (actin) after 7 days in culture [B] (20X objective, scale bar: 150 µm). Human fibroblasts stained with DAPI (nuclei) and RFP (actin) after 1 day in culture [C] (4X objective, scale bar: 650 µm). Human fibroblasts proliferated over a 3D-printed disc [D] & mesh [E] after 10 days in incubation (4X objective, scale bar: 650 µm). Spheroid of human fibroblasts embedded in Collink.3D™ 50 after 7 days incubation [F] (4X objective, scale bar: 650 µm). All images were taken with a EVOS 7000 microscope.

Storage and handling

- Collink.3D™ 50 is shipped in a temperature-controlled package. Upon receipt, the product should be stored at 2 °C to 8 °C, protected from light.
- Unless cured, the product should not be frozen or heated above room temperature.
- Avoid heating the product during the curing/printing process.
- Expiration date is indicated in the provided certificate of analysis and is applicable only when the product is handled and stored as directed.
- **For R&D use only. Not intended for human use.**

* (1) (2) (3) Data on File at CollPlant.